Exhibit **K-25**

Project No._____ Appl. No. 09/558,421

Book No._____ 12/19/94 **141**

age N .____

PMC9 Revisited:

Purpose : To repeat the exercise again with pmc9 for fidelity

Just : to titrate the amount of template. I used 100 - 200 pg / 35 cycles / 3 step, 3' extension & lot of mispriming was obvious

Judy has used earlier less template / 30 cycles / 2 step, less of other products / 5' extension

so tried 0, 10, 25, 50, 100 & 200 pg of starting template / 5' extension / 2 step

with 1 U of enzyme product yield is very little - but with 2 U of Taq, quintagood product yield was obtained.

so tried with both 1U & 2U of Taq alone

To start with, in this expt Taq & DV wasnt included

Conditions :

    16 x buffer K-T.   94°, 30"
    200 μM dNTP
    .4 μM primers (non-dw)   30 (94°, 30", 60°, 5')
    2 mM Mg
    Template 0 - 200 pg
    enzyme 1 & 2 U   — prepared a premix
                             12 × 45 μl
/ tubes: 1 - 12   1U
          13 - 24   2U   — added 5 μl of different
                                amount of template

sed & Understood by me,   Date 1/9/95   Invented by

Record d by   12/19/94

K. A Baraman

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Tag

0   10   25   50   100   200 pg   MyM
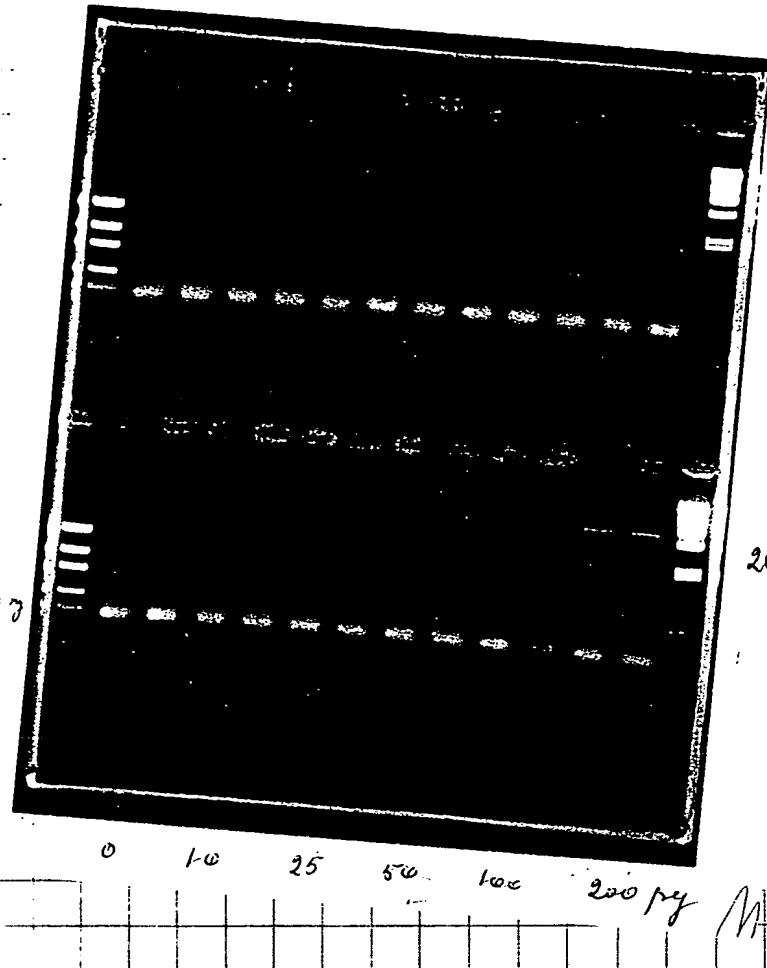
Result

200 pg / 1u enzyme ✓

25 pg up / 2u product
obtained but
rather low
yield even with
200 pg of starting
template.

~ 5 - 10 ng / 8 µl

= 31.25 - 62.5 ng /
from 200 pg temp

~ 300 fold ampl

~ 8 doublings !

— ✳ Try 50/200 pg } 2u   200 pg — 1u

— Cycles increase to 35 atleast

— go up to 50, 60 —

— Cycling regime looks ok — gives cleaner product
— no mispriming seen

Witnessed & Understood by me,   Date 1/9/95

Invented by   Date 12/20/94

Recorded by   A. Sitaraman